REMARKS

The disposition of the claims is set forth below:

Disposition of Claims

4)☒ Claim(s) *20,49,51,57,59 and 65-71* is/are pending in the application.
4a) Of the above claim(s) _____ is/are withdrawn from consideration.
5)☐ Claim(s) _____ is/are allowed.
6)☒ Claim(s) *20,49,51,57,59 and 65-71* is/are rejected.
7)☐ Claim(s) _____ is/are objected to.
8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Claims 1-19, 21-48, 50, 52-56, 58, and 60-64 are canceled without prejudice or disclaimer.

New Claim Rejections under 36 U.S.C. Section 103

Claims 20, 49, 51, 57, 59, 65-70 and 71 are rejected under 35 U.S.C. 103(a) as being unpatentable over Feld et al (US patent no 7294333, dated 11/13/2007, filed on 10/20/2000), Lee et al (Molecular Therapy, 2001, 857-866, IDS, hereafter Lee 1), Lee et al. (USP 7,494,644, dated 2/24/2009, effective filing date 11/7/2002, art of record, hereafter Lee 2), and Qu et al (Circulation res. 2001, 89:e8-14, IDS).

In our response to the Final Office Action, Applicants amended independent claims 20 and 65 to include the negative limitation that the MSC are transfected with a nucleic acid encoding HCN channels but NOT with a nucleic acid encoding connexins, as is required by Lee.

Lee was discussed at the interview in the course of addressing the Advisory Action. Lee teaches the use of mesenchymal stem cells that have been genetically engineered to overexpress connexins such as Cx43 in order to enable the transfected cells to form gap junctions with cardiomyocytes. Connexin 43 (Cx43) is the major gap junction protein in the ventricular myocardium. Lee's requirement that the implanted MSCs overexpress recombinant connexin teaches away from currently amended claims 20 and 65, and claims that depend there from,

which include the negative limitation that the MSC are not transfected with a nucleic acid encoding connexins.

The Examiner indicated at the interview that the Lee reference could be removed if Applicants provide specific support in the application as filed for the negative limitation in the pending independent claims 20 and 65, which states:

, and wherein the MSC is not incorporated with a nucleic acid which encodes a connexin.

As was discussed in the interview, by "not incorporated with a nucleic acid" means that the MSC are NOT transfected with a nucleic acid encoding a connexin; however, the MSC are "incorporated with (i.e. transfected with) a nucleic acid encoding an HCN2 channel.

Paragraph [0133] of the present application states:

> *[0133]...The cardiac gap junctions are composed of some combination of three subunit proteins: connexin43 (Cx43), and/or Cx40, and/or Cx45. The major goals of this phase are to determine the types of connexins expressed and functioning in stem cells transfected with pacemaker genes for a biological pacemaker, and stem-cell derived cardiogenic cell lines which will be used for cardiac repair. One may also determine the ability of these cell types to form gap junctions with normal adult cardiac myocytes from nodal, atrial, Purkinje, and ventricular myocardium. If necessary or desirable, one can investigate transfection of either preparation with relevant connexin genes.* Because both ionic permeability (assayed by measuring gap junctional conductance) and permeability to physiologic second messengers (assayed by larger molecular weight fluorescent dye permeation) are important, both measurements will be made in our experimental protocols.

To summarize:

- Gap junctions are composed of connexins
- MSCs transfected with the pacemaker gene used in the present invention need to be able to form gap junctions with cardiomyocytes, which requires that they express one or more connexins.
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- Applicants determined that normal MSCs that are NOT transfected with (i.e. "incorporated with") a connexin gene are nonetheless able to form gap junctions with cardiomyocytes relying only on the expression of endogenous connexins. Evidence of both ionic coupling and dye transfer between MSC and cardiomyocytes indicating the formation of gap junctions is described in FIG. 3.

[0023] FIG. 3A-C: coupling and ionic and dye transfer between stem cells and between a stem cell and a canine cardiomyocyte (ventricle). A: light micrograph and fluorescence images of dye transfer between stem cells. B: light micrograph and fluorescence images of dye transfer between a stem cell and a canine cardiomyocyte. C: graph representing ionic transfer between a stem cell and canine cardiomyocyte.

Applicants have also provided definitive proof in the previously submitted Rosen declaration that nontransfected hMSCs can form gap junctions with cardiomyocytes *in situ* using immunostaining Circ Res 2004; 94:952-959; and they showed that hMSCs express Cx40 and Cx43 using immunostaining Valianus J Physiol. 555.3, 2004 p 617. Copies are attached. These were described in Michael Rosen's declaration.

- Paragraph [0133] indicates that MSC transfected with one or more connexins COULD be used, but is not required, thus supporting the negative limitation with the language: *"**If** necessary or desirable, one can investigate transfection of either [stem cell] preparation with relevant connexin genes."*

- *Applicants discovery that MSC can form gap junctions without being transfected to overexpress connexins is a significant improvement.*

Applicants therefore submit that the pending claims are not obvious over the teachings of Lee which teaches that MSCs must be transfected to overexpress connexins in order to form gap junctions with target cardiac cells. In sharp contrast to Lee, the inventors of the present application discovered that undifferentiated hMSCs are able to form gap junctions without being genetically modified to overexpress connexins. The hMSCs in the pending claims are genetically modified with HCN ion channels to generate a pacemaker current, but unlike Lee the hMSCs are not engineered to overexpress connexins.

While Applicants are willing to add the negative limitation that the MSC used in the invention are NOT transfected with connexin genes, they reserve the right to pursue claims to the use of MSC that are transfected with a gene or genes encoding connexins in a divisional application.

Applicants ask the Examiner to withdraw the rejection of the pending claims for obviousness because the Lee reference teaches away from the present invention.

Wang et al

In a previous Nonfinal OA, the examiner had cited Wang et al as part of an obviousness rejection that was withdrawn in view of the Rosen declaration. The Examiner stated:

> Wang et al provided guidance with respect to administration of MSC in the heart shows growth potential in a myocardial environment and indicated the formation of gap junctions (see abstract and Figure 6).

The Examiner indicated that even if he withdraws his obviousness rejection (above) by disqualifying the Lee reference, he would consider another obviousness rejection that included the Wang reference. The Examiner invited Applicants to address Wang in this response.

The Wang 2000 reference provides only suggestive morphological data that injected stem cells form gap junctions with myocytes. It does not provide proof that there is functionality, i.e. functioning ion channels between stem cells and myocytes that can conduct current or transfer molecules (as evidenced, for example by dye transfer). The Wang studies do not provide an independent marker for the stem cells and myocytes within the image they claim demonstrates the presence of gap junctions between stem cells and myocytes (figure 1E) in the 2002 paper and figure 6 in the 2000 paper. Incidentally, these two figures are the same figure in both papers. Therefore a person of skill in the art would not rely on Wang as proof that MSC can form gap junctions with cardiomyocytes, or any other cell. Importantly, the Lee reference has an effective date one year later than Wang (Lee claims priority to U.S. provisional application Ser. No. 60/337,352, filed Nov. 8, 2001). Even though Lee comes after Wang, Lee disregards the teachings of Wang and nonetheless still teaches the necessity of transfecting the MSC with connexins to achieve gap junction formation with target cells. At the very least, there is therefore a conflict of opinion among experts as to whether MSC could form functioning gap junctions without being transfected with connexins.

Applicants were the first to provide definitive evidence that undifferentiated MSCs are able to form fully functional low resistance junctions with cardiomyocytes, and naturally couple to heart cells *in situ* via gap junctions that permit dye transfer between stem cells and cardiomyocytes in *in vivo* studies that show the presence of stem cells within the myocardium and the ability to pace the heart demonstrate functionality (Valiunas et al2004; Potapova et al., 2004).

Therefore, Applicants respectfully submit that the present application is not obvious and the claims are in condition for allowance. Favorable consideration is respectfully requested. If any unresolved issues remain, it is respectfully requested that the Examiner telephone the undersigned attorney at (703) 622-6528 so that such issues may be resolved as expeditiously as possible.

To the extent necessary, a petition for an extension of time under 37 C.F.R. § 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 505429 and please credit any excess fees to such deposit account.

Respectfully Submitted,

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August 04, 2011 /Judith Evans, 38874/

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